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RESEARCH ARTICLE

Free radical scavenging activity of various extracts of whole plant of *Pavetta indica* (Linn) : An *in-vitro* evaluation

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Abstract: The study was designed to examine the *in vitro* antioxidant activities of various extracts of aerial parts of *Pavetta indica.* The antioxidant activity was evaluated by superoxide anion scavenging activity and nitric oxide scavenging activity with reference standard Quercetin, and ascorbate respectively and estimate the amount of total phenol. The methanolic extract of *Pavetta indica* was found to more effective in the superoxide anion scavenging activity. The IC_{50} of the methanolic extract of *Pavetta indica* and quercetin were found to be 190µg/ml and 60µg/ml respectively. An IC_{50} value was found that methanolic extract of *Pavetta indica* is more effective in scavenging nitric oxide radical than that of ethyl acetate and petroleum ether extract. But when compare to the all the three extracts with ascorbate (standard), the methanolic extract of the *Pavetta indica* showed the similar result. In addition, the methanolic extract of *Pavetta indica* was found to contain noticeable amount of total phenols, which play a major role in controlling antioxidants. It is concluded that a aerial parts of methanolic extract of *Pavetta indica,* which contains large amounts of phenolic compounds, exhibits high antioxidant and free radical scavenging activities. These in vitro assays indicate that this plant extracts is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Key words: *Pavetta indica*, *In vitro* antioxidant, Nitric oxide scavenging, Superoxide anion, Total phenol.

INTRODUCTION:

Antioxidants are habitually utilized in oils and fatty foods to retard their auto oxidation. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxy anisole (BHA), have restricted utilise in foods as they are suspected to be carcinogenic. Hence, the importance of search for natural antioxidants has greatly increased in the recent years[1]. Ethnomedical survey contains a huge number of plants that can be used against diseases, in which reactive oxygen species and free radical play important role. There is a plethora of plants that have been found to possess strong antioxidant activity[2]. Current reports specify that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases[3]. So, many researchers have focused on natural antioxidants and in the plant kingdom numerous crude extracts and pure natural compounds were previously reported to have antioxidant properties.

Pavetta indica Linn. belongs to the family Rubiaceae. It is widely distributed from the Andaman Islands, India and the north-western Himalayas to southern China and southwards throughout Malaysia to northern Australia. A shout bushy shrub 0.6-1.2 m high; bark thin, smooth, yellowish; young branches terete, glabrous. Leaves 7.5-15 by 2.5-6.3 cm, membranous, variable in shape and size, elliptic - oblong or elliptic - lanceolate, sometimes obovate - oblong, obtuse, acute or acuminate, glabrous on both sides, base tapering; main nerves 8-10 pairs; petioles 6-13 mm long; stipules connate, triangular, acute, thin, deciduous. Flowers white, odourous, in terminal sessile corymbose pubescent cymes; pedicles 4-6 mm long, densely pubescent; bracts broad, membranous, the lower copular; buds oblong- clavate. Calys densely pubescent, 3mm long; tube narrowly campanulate; teeth 1.25 mm long, triangular, acute, slightly reflexed at the tip. Corolla - tube 13 mm long; lobes 6-8 by 2.5 mm, linear - oblong, subacute. Style white, glabrous or nearly so; stigma green, narrowly clavate, puberulous. Fruit 6-14 mm diameter, glabose, black, smooth. The entire plant used medicinally as a bitter tonic, diuretic, inflammation, rheumatism, jaundice and ulcer[4]. In the indigenous system of medicine, it is reported that the decoction of the leaves are used to relieve haemorrhoidal pain, as a lotion for nose, analgesic, antipyretic, appetizer and the ulceration of mouth[5,6]. In literature, it has been reported as an antibacterial, anti-

viral and antimalarial[7]. *P. indica* leaves are used in the treatment of liver disease, pain from piles, urinary diseases and fever[8]. It is a medicinally important plant having antiinflammatory activities[9] (Mandal *et al*., 2003). Golwala *et al*. (2009) reported analgesic activity[10], antidiabetic activity[11], antimicrobial[12] activity of leaf extract of *P. indica.* Its root extract also have diuretic and purgative activity[13] (Kumar, 2006). The leaves and roots are employed in the preparation of poultices for boils and itches; decoctions of leaves are used as a lotion for ulcerated nose and for heamorrhoids. Root is used for anticephalagic. Leaf is used in haemorrhoidol pain and ulcerated nose. Wood is used as antirheumatic. Fruits are used as anthelmintic[14-17]. The phytochemicals produced by the plants for their self protection have been demonstrated to protect human against a number of diseases. The leaves contain carbohydrate, glycosides, phytosterols, saponins, flavonoids and alkaloids. However, no data are available in the literature on the antioxidant activity of aerial parts of *Pavetta indica* (Linn). Therefore we undertook the present investigation to examine the antioxidant activities of various extract of aerial parts of *Pavetta indica* (Linn) through various *in vitro* models.

Materials and Methods

Collection and Identification of Plant materials

The aerial parts of *Pavetta indica* (Linn), were collected form kalakkadu, Tirunelveli District , Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The aerial parts of *Pavetta indica* (Linn), were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The above powered materials were successively extracted with Petroleum ether (40-60^{0}C) by hot continuous percolation method in Soxhlet apparatus[18] for 24 hrs. Then the marc was subjected to Ethyl acetate (76-78^{0}C) for 24 hrs and then mark was subjected to Methanol for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Evaluation of antioxidant activity by *in vitro* techniques:

Superoxide anion scavenging activity[19]

Superoxide radical (O_2^-) was generated from the photoreduction of riboflavin and was deducted by nitro blue tetrazolium dye (NBT) reduction method. Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne et al (1975)[19]. The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 ml of EDTA (0.1M EDTA), 0.05 ml riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up where in DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Ascorbate was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

Nitric oxide radical scavenging activity[20]

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions. The reaction mixture (3ml) containing 2 ml of sodium nitroprusside (10mM), 0.5 ml of phosphate buffer saline (1M) were incubated at 25^{0}C for 150 mins. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33%) and allowed to stand for 5 min for completing diazotization. Then 1 ml of naphthylethylene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 30 mins. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess Illosvery reaction at 540 nm.

Total phenol[21]

The measurement of total phenol is based on Mallick and Singh (1980)[21]. To 0.25g of sample, added 2.5 ml of ethanol and centrifuged at 2^{o}C for 10 mins. The supernatant was preserved. Then, the sample was re-extracted with 2.5 ml of 80% ethanol and centrifuged. The pooled supernatant was evaporated to dryness. Then, added 3 ml of water to the dried supernatant. To which added 0.5 ml of Folins phenol reagent and 2 ml of sodium carbonate (20%). The reaction mixture was kept in boiling water bath for 1 min. the absorbance was measured at 650 nm in a spectrophotometer.

Results and Discussions

Superoxide anion scavenging activity

Superoxide Percentage scavenging of superoxide anion examined at different concentrations of petroleum ether extract of *Pavetta indica* (125, 250, 500, 1000 µg/ml) was depicted in table 1.The percentage scavenging of superoxide radical surged with the enhanced concentration of plant extract. The maximum scavenging activity of plant extract and quercetin at 1000 µg/ml was found to be 58.24% and 98.01% respectively. The IC_{50} value of plant extract and quercetin was recorded as 520µg/ml and 60µg/ml respectively.

Percentage scavenging of superoxide anion examined at different concentrations of ethyl acetate extract of *Pavetta indica* (125, 250, 500, 1000 µg/ml) was depicted in table 2. The percentage scavenging of superoxide radical surged with the enhanced concentration of plant extract. The maximum scavenging activity of plant extract and Quercetin at 1000 µg/ml was found to be 71.46% and 98.01% respectively. The IC_{50} value of plant extract and Quercetin was recorded as 435µg/ml and 60µg/ml respectively.

Percentage scavenging of superoxide anion examined at different concentrations of methanolic extract of *Pavetta indica* (125, 250, 500, 1000 µg/ml) was depicted in table 3. The percentage scavenging of superoxide radical surged with the enhanced concentration of plant extract. The maximum scavenging activity of plant extract and Quercetin at 1000 µg/ml was found to be 80.45% and 98.01% respectively. The IC_{50} value of plant extract and Quercetin was recorded as 190µg/ml and 60µg/ml respectively.

Table 1: Effect of Petroleum ether extract of *Pavetta indica* on superoxide anion scavenging activity method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Quercetin)
1	125	25.64 ±0 .058	73.81 ± 0.006
2	250	41.18 ± 0.056	91.31 ± 0.011
3	500	50.34 ± 0.024	92.99 ± 0.024
4	1000	58.24 ± 0.015	98.01 ± 0.012
Results		**IC_{50} = 520 µg/ml**	**IC_{50} = 60 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 2 : Effect of ethyl acetate extract of *Pavetta indica* on superoxide anion scavenging activity method:

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Quercetin)
1	125	22.43 ± 0.018	73.81 ± 0.006
2	250	39.58 ± 0.054	91.31 ± 0.011
3	500	55.76 ± 0.032	92.99 ± 0.024
4	1000	71.46 ± 0.046	98.01 ± 0.012
Results		**IC_{50} = 435 µg/ml**	**IC_{50} = 60 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 3 : Effect of methanolic extract of *Pavetta indica* on superoxide anion scavenging activity method:

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Quercetin)
1	125	43.26 ± 0.042	73.81 ± 0.006
2	250	60.48 ± 0.026	91.31 ± 0.011
3	500	74.66 ± 0.048	92.99 ± 0.024
4	1000	80.45 ± 0.026	98.01 ± 0.012
Results		**IC_{50} = 190 µg/ml**	**IC_{50} = 60 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 4: Nitric oxide scavenging activity of Petroleum ether ext ract of *Pavetta indica*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	21.46 ± 0.026	26.87 ± 0.076
2	250	32.68 ± 0.045	30.30 ± 0.054
3	500	43.94 ± 0.032	60.64 ± 0.022
4	1000	51.04 ± 0.056	75.23 ± 0.014
Results		**IC_{50} =1005 µg/ml**	**IC_{50} = 410 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 5: Nitric oxide scavenging activity of ethyl acetate extract of *Pavetta indica*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Ascorbate)
1	125	21.35 ± 0.042	26.87 ± 0.076
2	250	32.38 ± 0.024	30.30 ± 0.054
3	500	42.88 ± 0.038	60.64 ± 0.022
4	1000	55.68 ± 0.016	75.23 ± 0.014
Results		**IC_{50} = 905 µg/ml**	**IC_{50} = 410 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 6 : Nitric oxide scavenging activity of methanolic extract of *Pavetta indica*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	45.48 ± 0.024	26.87 ± 0.076
2	250	52.14 ± 0.018	30.30 ± 0.054
3	500	64.32 ± 0.038	60.64 ± 0.022
4	1000	72.24 ± 0.044	75.23 ± 0.014
Results		**IC_{50} = 210 µg/ml**	**IC_{50} = 410 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 7: The total phenolic content of various extracts of whole plant of *Pavetta indica*

S. No	Extracts	Total phenol content (mg/g of Catechol) (±SEM)*
1	Petroleum ether extract of *Pavetta indica*	1.64 ± 0.010
2	Ethyl acetate extract of *Pavetta indica*	2.12 ± 0.021
3	Methanolic extract of *Pavetta indica*	5.16 ± 0.026

*All values are expressed as mean ± SEM for three determinations

Nitric oxide scavenging activity

The reduction of nitric oxide radical by the petroleum ether extract of *Pavetta indica* and ascorbate was illustrated in Table 4. The maximum scavenging activity of petroleum ether extract and ascorbate at 1000 µg/ml were found to be 51.04 % and 55.23% respectively. The IC_{50} value of petroleum ether extract and ascorbate were recorded as 1005µg/ml and 410µg/ml respectively.

The reduction of nitric oxide radical by the ethyl acetate extract of *Pavetta indica* and ascorbate was illustrated in Table 5. The maximum scavenging activity of ethyl acetate extract and ascorbate at 1000 µg/ml were found to be

52.47% and 55.23% respectively. The IC_{50} value of ethyl acetate extract and ascorbate were recorded as 905µg/ml and 410µg/ml respectively.

The reduction of nitric oxide radical by the methanolic extract of *Pavetta indica*and ascorbate was noted to be concentration dependent and was illustrated in Table 6. The maximum scavenging activity of methanolic extract and ascorbate at 1000 µg/ml were found to be 72.76% and75.23% respectively. The IC_{50} value of methanolic extract and ascorbate were recorded as 210µg/ml and 410µg/ml respectively.

Total phenol

The total amount of phenolic content of various extract of whole plant of *Pavetta indica* was present in Table 7.

Based on the result the methanolic extract of *Pavetta indica* was found higher content of phenolic components than that of petroleum ether and ethyl acetate extract of *Pavetta indica.*

Conclusion

The present study was clearly indicated the methanolic extract of *Pavetta indica* showed strong antioxidant activity by inhibiting super oxide anion scavenging activity, nitric oxide radical scavenging activities when compared with standard quercetin and ascorbate. In addition, the methanolic extract of *Pavetta indica* was found to contain noticeable amount of total phenols, which play a major role in controlling antioxidants. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

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